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III—THE FUNGI CAUSING MILDEW IN COTTON GOODS

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IV—THE INHIBITORY ACTION OF CERTAIN SUBSTANCES ON THE GROWTH OF MOULD FUNGI

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FIG. 1

Colonies of mildew fungi and bacteria developing on sterilised wort agar. Each glass dish was inoculated with 4 sq. cm. of a clean grey cloth.

The small white spots towards the top-right are colonies of bacteria. The black spot at the extreme right is a growth of *Cladosporium*.

The large white mass is a colony of *Fusarium*.

The powdery black mass is a colony of *Aspergillus niger*.

III—THE FUNGI CAUSING MILDEW IN COTTON GOODS

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I—SUMMARY

In connection with the routine examination of mildew cases, and with the testing of antiseptics suitable for use in sizing, it has been necessary to isolate a considerable number of mildew fungi in pure culture. This paper gives a brief summary of the occurrence and characteristics of the types thus isolated, and of the difficulties encountered in a preliminary classification.

The majority of species belong to the two genera *Aspergillus* and *Penicillium*, but *Fusarium* and other *Fungi Imperfecti* are of common occurrence. It is shown that the raw cotton itself is the principal source of infection. Only a proportion of the fungi are capable of producing stains on cloth, and a still smaller proportion capable of causing tendering under ordinary conditions of exposure.

II—OCCURRENCE OF MILDEW FUNGI

The term mildew as understood in the cotton industry applies to any fungal growth producing an undesirable effect—usually a discoloration—on cloth or yarn. Mildew may occur at all stages of manufacture; in raw cotton, in yarn, in unbleached cloth, and in dyed or printed goods. It causes most loss, however, in grey (unbleached) cloth, and claims for mildew in goods of this class shipped to Eastern markets are of regular occurrence. This is said to be especially so in times of trade depression, when goods are liable to be kept longer in storage.

A more complete general statement of the mildew problem has been given by Bright, Morris, and Summers.[6] The mildew collection described in the present paper consists in part of cultures isolated by these workers.

III—SOURCE AND EXTENT OF INFECTION

All cotton goods are infected with mildew organisms, since spores are continually being deposited from the air. Cloth and yarn in the grey state have in addition the heavy infection present in the raw cotton. Some species of mildew (e.g. *Aspergillus niger*[12a]) are traceable right back to the cotton field, where they cause diseases of the boll.

The heavy infection of an odourless and apparently clean grey cloth is illustrated in Fig. 1, where "colonies" of moulds and bacteria growing on the medium in each circular dish have developed, after several days, from 4 sq. cm. of the fabric teased out into warp and weft and scattered over the medium. A somewhat similar experiment is recorded by Osborn[12].

The extent of infection cannot easily be expressed by a numerical value, since the fungi are present not only as spores but also as mycelium (the thread-like stage of the fungus) in and among the cotton hairs, and either form is capable of giving rise to a fungus colony. The same difficulty is encountered by workers on soil microbiology (Waksman[18]).

Mildew spores and mycelium readily stain with the dyestuff Cotton Blue, whilst cotton hairs do not. A technique based on this[4] greatly simplifies the microscopic examination of a fabric suspected of mildew.* A more rapid

*Warm the specimen in lactophenol (lactic acid 1, phenol 1, glycerin 2, distilled water 1) on a glass slide for 1 minute. Transfer to cotton blue (1% aqueous solution) and warm several minutes. Wash in water, then warm with lactophenol until as much as possible of the colour is removed. Mount in lactophenol.

but somewhat less satisfactory preparation may be made by staining with warm picro-aniline blue and washing with water.

IV—METHODS OF CONTROL

Since infection cannot be avoided, methods of control of mildew are based on the prevention of further growth, and it cannot too often be emphasised that the principal factor is the amount of moisture present. The available evidence in the literature indicates that in cloth with 8% moisture regain or less, no further development of mildew will occur.[1] Samples of three different grey cloths kept in the laboratory with moisture regains of 8 to 10% confirm this; after three months' incubation at 25° C., cloths with 8·5, 8·9, and 9·0% regain showed no mildew, whilst cloths with 9·5 and 10·0% showed very slight growth.

In practice, however, it is not always possible to ensure this low moisture regain, and the use of an antiseptic becomes necessary. Mildew organisms show very large specific differences in their behaviour towards antiseptics,[11] and it is largely because of this wide variation that it has been considered worth while to classify the organisms that are most frequently encountered. The most resistant of the species commonly occurring have been chosen for exhaustive antiseptic tests, the results of which are recorded in the following memoir.

A third possible method of suppressing mildew growth is to maintain a high concentration of carbon dioxide in the storage atmosphere, though this is not practicable for cotton goods, except perhaps in special instances. Preliminary experiments have shown that in an equal mixture of air and carbon dioxide the growth of certain cotton mildews is very considerably suppressed, whilst air containing even 25% carbon dioxide exerts an appreciable effect. These figures may be compared with the results of Bavendamm[2] who found that with 32 species of wood-destroying fungi, 19% excess carbon dioxide checked growth, and 80% completely inhibited growth; or with those of Brown,[7] whose test fungi were appreciably affected by 20% carbon dioxide and almost all suppressed by 60 per cent.

V—DISTRIBUTION OF VARIOUS TYPES OF MILDEW FUNGI

The list given in Table I shows the source of a number of fungi that have been isolated. The classification is by no means complete, and represents a preliminary attempt to sort the collection into groups of closely related types, rather than to attain an exact identification (not always possible) of species. The principles on which the classification is based are discussed in greater detail in Section VII. All the laboratory record numbers are given, and pure cultures are being maintained at the Shirley Institute.

It will be seen that there is little correlation between source and species. *Aspergillus niger*, for example, occurs in raw cotton, as an infection of the air of the mill, and in cloth mildewed at home and abroad. It has frequently been observed both in grey cloth and as a frequent cause of spotting in alizarine prints. This indicates the impossibility of using the identification of the causal organism as a means of tracing the stage at which mildew occurred.

There are, however, a few general remarks that can be made with respect to distribution. The two genera, *Aspergillus* and *Penicillium*, are the most abundant. *Penicillium* species tend to grow at lower temperatures than *Aspergillus* species, and hence are more frequent in mildew developing in

this country, whereas *Aspergillus* species are more common in mildew developed in hot climates. In this connection it is interesting to notice that Waksman[17] in a study of the fungal flora of American soils, found that in the warmer south *Aspergillus* was the predominant genus, whilst *Penicillium* and *Mucorales* were found more extensively in northern states.

The degree of humidity also determines to some extent the types that predominate. The series of fungi forming the *Aspergillus glaucus* group is of common occurrence in cloth of moisture content near the limit of growth. This agrees with an observation of Thom and Le Fevre[16] that in corn meal this group is the first to develop as the moisture content rises above 12·5–13·0 per cent. Fortunately, the *Aspergillus glaucus* group is very susceptible to most antiseptics.

VI—THE EFFECTS OF MILDEW

The effects may be summarised briefly as (1) musty smell, (2) coloured stains, (3) production of acid (or alkali), and (4) tendering.

(1) *Smell*—A musty odour usually accompanies the development of mildew in a confined space, and often precedes any visible effect on the cloth. The smell soon disappears if the cloth is freely exposed to air, and it is a common practice to open up and replait cloth that has developed a musty smell during storage.

(2) *Stains*—This is by far the most important effect, and is the cause of the majority of mildew claims. Two sorts of staining may be noted. In the first, the discoloration is due to the formation of masses of coloured spores; this type usually occurs under moist conditions, and such stains can sometimes be removed by brushing. In the second type of stain, the colour is due to pigment formation by the fungal hyphæ or the perithecia of certain mildews, or to coloured products formed by the action of the mildew on starch and other food substances present.

In dyed goods the dye may be changed in colour by acids or other substances produced by the mildew fungus—see (3).

Table I indicates those species in culture that produced definite stains when inoculated on strips of sterile grey cloth. The strips of cloth were placed in test tubes, the lower end of the cloth dipping in water; the tubes were then plugged with cotton wool, sterilised, and then inoculated and incubated at 25° C. in the usual manner.

It should be noted that the colour of the stain may be affected by various factors, such as the reaction of the cloth, the presence of other organisms, and certain chemical substances. The brown stain produced by *Aspergillus terreus* 48, for example, is greatly intensified in the presence of traces of zinc chloride.

Where "nil" appears in Table I in the column headed "Cloth Stains" the species failed to give stains under the conditions of test; it by no means follows that none would cause stains under the varying conditions met with in actual practice. Some in fact, are known to do so, e.g. certain *Aspergillus glaucus* spp. sometimes produce orange or yellow stains due to coloured perithecia.

(3) *Production of Acid from Starch*—This is closely connected in its commercial aspects with (2) since its principal importance lies in its connection with the spotting of coloured fabrics, such spotting being usually traceable to acid produced by mildew. Witz[20] observed in 1876 that the spots had an acid reaction, but considered that the acidity was "probably due to the

formation of lactic and butyric acids in fermentation" by bacteria present. Since that time much work has been published on the production of gluconic, oxalic, and citric acids by *Aspergillus niger* and other fungi.

The great variation in acid production by different species is shown in Table I. This gives the results obtained by growing a number of pure cultures (at 25° C. for one week) on a 3% maize starch paste coloured by litmus. Some species, such as *Cladosporium* 15, and *Aspergillus versicolor* 45, produce no appreciable acid. *Aspergillus niger* (e.g. sp. 169) on the other hand, is one of the most vigorous acid producers and it is not surprising that spotted prints have usually yielded this type as the predominant infection.

Similar spotting may be produced by a nutrient sugar solution on which *Aspergillus niger* has been growing for some days, or by 0·2% oxalic acid or by 0·3% citric acid.

(3a) *Production of Alkali*—Many fungi when grown on a nitrogenous substratum are capable of ammonia production. It is, therefore, not surprising to find that a number of mildew fungi when grown on unbleached cotton produce an appreciable alkalinity.

In unbleached cotton goods containing starch, however, alkalinity due to fungi is more rare, either because the protein is not attacked when there is an abundant supply of starch, or because alkali production is masked by acid production.

Only a few of the fungi examined [*Cladosporium*, *A. versicolor*, *Penicillium* series (a)] actually cause alkalinity on sized cloth and such species naturally do not include those that form considerable acid from starch. On raw yarn both *Cladosporium* 15 and *Aspergillus flavus* 35 produce alkali; on grey cloth only the former does so.

It should be noted that in practice grey cloth exposed to high humidities rapidly becomes alkaline. Such alkalinity, however, is principally due to the bacteria that commonly accompany mildew and not to mildew fungi themselves.

Table I

Mould Fungi isolated from Cotton Goods.

The reference numbers are those assigned to the cultures maintained at the Shirley Institute. The division of related species into sub-groups is indicated by separate headings.

Species		Source	Cloth Stain under Culture Conditions	Acidity from Starch	Cellulose Decomposition
		ASPERGILLUS			
A. niger group—					
	8	Yarn		Strong	Moderate
	133B	Cloth mildewed abroad		Slight	
	133C	,, ,, in England	Brown	Slight	
	133D	Raw cotton (Queensland)			
	160E	,, ,, (Sakel)			
	169	Cloth mildewed in England	Nil	Strong	
A. glaucus group—					
A. repens	1	Yarn		Nil	Nil
	26	,,			
	56	Cloth mildewed abroad			
	120	Yarn (Egyptian)	Nil		
	129	,, ,,			
A. ruber	23	,,			Nil
	55	Cloth mildewed abroad		Nil	
	115	,, ,, ,,	Nil	Nil	
Allied to *A. chevalieri*	70	,, ,, ,,	Nil		
	80	,, ,, ,,	Nil		

Table I—Mould Fungi isolated from Cotton Goods—*cont.*

Species		Source	Cloth Stain under Culture Conditions	Acidity from Starch	Cellulose Decomposition
		ASPERGILLUS—*cont.*			
A. glaucus group (*cont.*)—					
	77	Cloth mildewed abroad	Nil		
	89	,, ,, ,,	Nil		
	90	,, ,, ,,			
Allied to *A. chevalieri*	106	,, ,, ,,	Nil		
	102	,, ,, in England	Nil	Nil	
	103	,, ,, ,,	Nil		
	157D	,, ,, abroad			
Unidentified	25	Yarn	Nil		Nil
	78	,,			
A. glaucus/fumigatus types	110	Cloth mildewed abroad	Nil	Nil	
	171	,, ,, in England	Nil	Nil	
	179	,, ,, abroad	Nil		
A. fumigatus	74	,, ,, ,,		Strong	Strong
A. fumigatus/nidulans types	71	,, ,, ,,	Nil		
	91	,, ,, ,,	Nil		
	92	,, ,, ,,			
A. nidulans	31	Raw cotton (Zaria)	Yellow/brown	Nil	Strong
A. versicolor group—					
	24	Yarn			
	45	Cloth mildewed abroad	Nil	Slight	Moderate
	46				
A. versicolor	60	Cloth mildewed abroad	Reddish/brown (faint)		
	76	,, ,, ,,	Reddish/brown (faint)	Slight	
	95	Yarn			
	172	Raw cotton	Nil	Nil	
	173	Flour	Reddish/brown (faint)	Nil	
	44	Cloth mildewed abroad	Reddish/brown (faint)		Moderate
	61	,, ,, ,,	Reddish/brown (faint)	Slight	
A. Sydowi	88	,, ,, ,,	Nil	Slight	
	105	,, ,, ,,	Brown		
	131	,, ,, in England	Brown		
	135	,, ,, ,,	Nil		
	167	Yarn		Nil	
	35A	Raw cotton (Queensland)		Slight	Moderate
	35B	Yarn (Texas)			
A. flavus	132	Raw cotton (Durango)		Slight	
	157C	Cloth mildewed abroad			
	157F	,, ,, ,,			
A. effusus	21	,, ,, ,,			
A. terreus	48	,, ,, ,,	Brown	Strong	Strong
A. Wentii	75	,, ,, in England	Yellow/brown	Slight	Moderate
	160A	Raw cotton (Sakel)			
A. ochraceus	73	,, ,, mildewed in England	Brown (faint)	Slight	Moderate
	73A	Cloth mildewed in England			
	162	,, ,, abroad	Red	Slight	
A. tamarii	28A	,, ,, ,,	Brown		Moderate
	28B	Raw cotton			
A. candidus Spp.	11	Yarn		Nil	Nil
	18	Dust		Nil	
	49	,,	Nil		
	52	,,			
	158	Yarn	Nil	Nil	
A. flavipes	175A	,, (Abassi)		Slight	
	175B	,, (American)		Slight	Strong
Unidentified	166	Cloth mildewed in England	Yellow/brown	Nil	
		PENICILLIUM			
	85	Air	Green (faint)	Nil	Nil
	87	Yarn (Egyptian)	Green/brown (faint)		
Series (*a*)—					
(?) Related to *P. candidofulvum*, Biourge	72	Cloth mildewed abroad			
	116	,, ,, in England		Nil	
	117	,, ,, ,,			
	130	Yarn (Egyptian)	Nil	Nil	Nil

Table I—Mould Fungi isolated from Cotton Goods—*cont.*

Species		Source	Cloth Stain under Culture Conditions	Acidity from Starch	Cellulose Decomposition
		PENICILLIUM—*cont.*			
	4	Cloth mildewed abroad	Green/yellow	Strong	Moderate
	17	Dust	Green/brown (faint)		Moderate
	42	Cloth mildewed in England	Yellow/brown	Strong	
Series (*b*)—	50	Yarn		Strong	
(?) Related to *P. chrysogenum* Thom.	69	Cloth mildewed abroad	Green (faint)		
	79	Yarn			
	101	Cloth mildewed in England	Nil		
	136	,, ,, abroad		Strong	
	176	Yarn		Slight	
	177	Cloth mildewed in England		Slight	
	68	,, ,, ,,	Green (faint)		
	94	,, ,, ,,	Green (faint)		Moderate
	97	,, ,, ,,			
Series (*c*)—	127	Yarn (Egyptian)	Green/yellow (faint)	Strong	
(?) Related to *P. citrinum* Thom.	144	Cloth mildewed abroad	Brown/yellow (faint)	Nil	
	148	Air		Slight	
	156	Cloth mildewed in England			
	157E	,, ,, abroad			
	2	Yarn	Green/yellow	Slight	Moderate
	98	,,			
Series (*d*)	86	Air		Nil	
	119	Yarn	Yellow (faint)	Strong	
	51	Cloth mildewed abroad	Nil		
	93	,, ,, in England	Nil		Nil
Series (*e*)	96	,, ,, abroad	Green/black (faint)		
	107	,, ,, ,,	Nil		Strong
Series (*f*)	82	,, ,, in England	Green (faint)		Moderate
(?) *P. Roqueforti.*					
Series (*g*)	109	,, ,, abroad	Nil	Nil	Moderate
	112	,, ,, ,,	Nil	Nil	
Series (*h*)	122	Yarn	Black (faint)	Nil	Strong
	123	Cloth mildewed in England		Nil	
Series (*j*)	114	Raw cotton	Green/black (faint)		Nil
(?) *P. chloro-leucon*, Biourge					
Series (*k*)	137	Cloth mildewed abroad	Green/yellow	Nil	Nil
Series (*l*)	140	,, ,, in England		Nil	Strong
Series (*m*)	154	Dust		Slight	Strong
Series (*o*)	168	Cloth mildewed in England	Nil	Strong	
Series (*p*)	157N	,, ,, abroad			
	OTHER	GENERA, INCLUDING FUNGI IMPERFECTI			
Mucor spp.—					
	6	Yarn			
Rhizopus arrhizus	34	Raw cotton (Queensland)		Strong	
	84	,, ,,		Nil	Nil
(?) *M. racemosus*	7	Size		Slight	
	19	Cloth mildewed abroad			
Not identified	29	,, ,, ,,			
	160D	Raw cotton (Sakel)			
	32	,, ,, (Zaria)	Nil		
	36	,, ,, (Queensland)		Strong	Nil
	38	,, ,, (Durango)	Nil	Strong	
Fusarium spp.	67	Yarn			
	146	Starch paste	Mauve	Slight	
	151	Raw cotton	Mauve	Strong	
	160B	,, ,, (Sakel)			
	83	Cloth mildewed in England	Yellow/black		Moderate
	108	,, ,, abroad			
Probably *Fusarium*	139	,, ,, in England	Mauve	Slight	
spp.	152	Air	Yellow	Slight	
	153	,,		Nil	
	15	Raw cotton (Zaria)	Black	Nil	Strong
Cladosporium herbarum	149	Yarn		Nil	
	161D	Cloth mildewed in England			
	30	,, ,, abroad			
Alternaria spp.	37	Raw cotton (Durango)	Purple/black	Nil	Nil
	5	Cloth mildewed in England	Green/black	Nil	Moderate
Stemphylium spp.	124	(?)	Green/black	Nil	Strong

Table I—Mould Fungi isolated from Cotton Goods—*cont.*

Species		Source	Cloth Stain under Culture Conditions	Acidity from Starch	Cellulose Decomposition
OTHER GENERA, INCLUDING FUNGI IMPERFECTI—*cont.*					
Macrosporium sp.	65	Yarn	Green/black	Nil	Moderate
Botrytis cinerea	10	,,			Moderate
Chætomium sp.	170	Raw cotton	Pink (faint)	Nil	Strong
Helminthosporium	141	Yarn	Pink/black	Slight	Strong
Dematium pullulans	66A	,,			Nil
	66D	Size			
Trichoderma sp.	33	Raw cotton		Nil	Moderate
	150	Yarn			Moderate
Monilia spp.	20	Cloth mildewed abroad	Green/black (faint)		Nil
	142	Yarn	Nil	Nil	Moderate
	157B	Cloth mildewed abroad	Green/brown (faint)		Nil
	160C	Raw cotton (Sakel)			
	163	Sago	Black/brown	Nil	
Actinomyces sp.	165	Cloth mildewed in England	Nil	Nil	
Unidentified	3	,, ,, ,,	Green/black	Nil	Strong
	9	Yarn			
	43	Cloth mildewed in England			Moderate
	53	Raw cotton	Black	Nil	
	58	Yarn	Nil		
	113	Cloth mildewed abroad			Nil
	126	Yarn	Nil	Nil	Nil
	128	,, (American)	Green/black	Nil	Strong
	149	,,	Green/black		
	155	Raw cotton	Black	Slight	Moderate
	174	Yarn			

(4) *Tendering*—Tendering or loss of strength is the result of cellulose being attacked by enzymes produced by the mildew fungi or by bacteria. Prolonged attack by certain species of mildew fungi may cause bad tendering, but usually the cellulose is either not affected at all, or is attacked so slowly that in practice tendering is not appreciable.

The examination of cotton hairs by the Congo Red method[5] readily shows up damage to the cuticle, even before any loss of tensile strength can be detected. Table II shows the effect of three fungi acting on wet cotton under pure culture conditions. It will be seen that in this experiment only *Aspergillus niger* has produced any significant effect on the tensile strength. Nevertheless, even the slow growing *A. versicolor* is capable of cellulose decomposition, and if the duration of the experiment had been longer this species might have produced appreciable loss of strength.

Table II
Tendering of 32's Texas Yarn

	Control	*A.versicolor* 45	*Rhizopus nigricans* 84		*Aspergillus niger* 8
		3 weeks	1 week	3 weeks	3 weeks
Ballistic test, work of rupture, gm/cm. per thread... ...	515	523	488	503	432
Single thread, breaking load, grams	240·1	247·9	240·1	234·8	196·3
Extensibility %	7·90	7·86	7·76	7·46	6·59
Congo Red examination ...	No damage	Slight	Slight	Slight	Considerably damaged

About 40 cultures were also grown on strips of filter paper sterilised in tubes containing 10 c.c. of the following medium recommended by Dubos[18].

Distilled water...	1 litre
Sodium nitrate, $NaNO_3$	0·5 g.
Potassium hydrogen phosphate, K_2HPO_4	1·0 g.
Magnesium sulphate, $MgSO_4,7H_2O$	0·5 g.
Potassium chloride, KCl	0·5 g.
Ferrous sulphate $FeSO_4$, $7H_2O$	0·01 g.

The degree of growth on the filter paper, though probably not an infallible criterion of cotton tendering power, gives a rough indication of the ability to decompose cellulose, and is indicated on Table I for the organisms tested.

The cellulose-destroying power of several of the fungi (e.g. *Aspergillus* 74, 31, sp. 43, etc.) has been confirmed by growth on cellulose-agar prepared in the usual way with filter paper reprecipitated from cuprammonium.

VII—IDENTIFICATION OF MILDEW FUNGI

The classification of the genera encountered is far from being completely worked out, and the worker who cannot devote considerable time to systematic work must be content with a general grouping of related species. Fortunately, the division into groups based on morphological features is found to agree fairly well with biochemical differences, and a rough classification is therefore sufficient for many purposes.

The structure and size of the sporing parts is the principal criterion in classification. Without careful control work, however, too great importance must not be attached to small differences in size, shape, or roughness of spores, since these details may vary slightly with the age of the culture and the medium.

Lactophenol is an excellent mounting medium, causing little or no distortion, and the original formula of Amann (already given, page 27) has been found quite satisfactory.

Aspergillus

This genus, thanks to the excellent monograph of Thom and Church[15], presents the least difficulty as regards classification. It can be divided into a number of natural groups; representatives of all these groups except the *Aspergillus clavatus* group are found on cotton goods, the *Aspergillus glaucus*, *versicolor*, *niger*, and *flavus* groups being the most common. Certain species referred to in this paper, and provisionally termed the *glaucus-fumigatus* group, have large oval spiny spores of the *glaucus* type, borne on conidiophores with terminal sterigmata of the *fumigatus* type.

A paper by Smith[13] describes a number of *Aspergillus* types found in cotton and gives descriptions which agree with those of Thom.

Pencillium

The classification of this genus is on a less firm basis. A number of species have been described by Thom,[14] but most of these do not appear to be common mildew forms.

In Westling's classification[19] the genus is divided into two groups, *Eupenicillium* and *Aspergilloides*, represented by the *camera lucida* drawings in Fig. 2. A summary of Westling's key is given by Mason,[10] but in this abridged translation identification appears to depend unduly on small differences in spore measurement and other characters subject to variation.

The large and well-illustrated monograph by Biourge[3] is the fullest account of the genus yet available, but gives no simple key to the identification of

species. Biourge's sub-division of *Eupenicillium* into *Bulliardium* and *Biverticillium*, and his emphasis on the zonation of cultures, require to be treated with caution, and should not be relied on for diagnostic purposes without considerable experience. On the other hand, the sugar-gelatin medium he describes under the name of "Raulin-Dierckx neutre" is invaluable in differentiating species by virtue of the remarkable range of colour production that it stimulates.

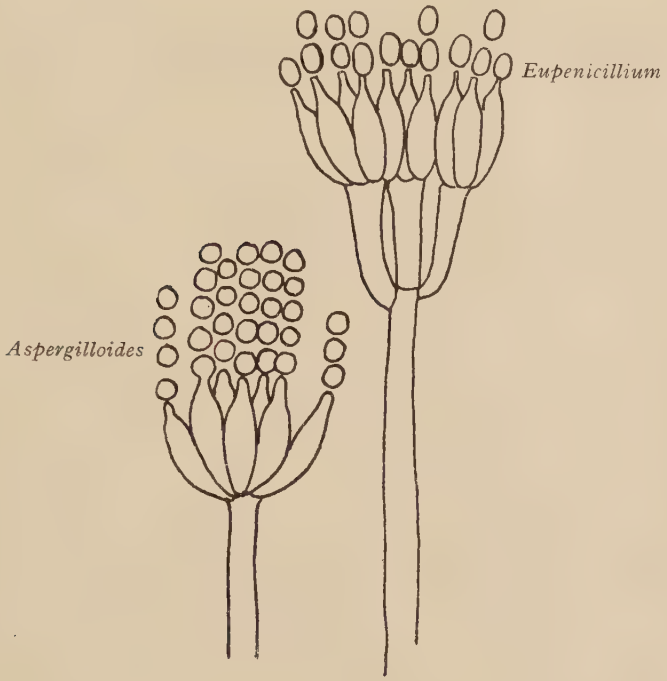

Fig. 2

In the present collection the *Penicillium* species have been provisionally grouped into series (*a*) to (*p*) of forms similar in general appearance and biochemical characters, though showing small individual differences, e.g. series (*b*) liquefies sugar gelatin media with the production of a yellow liquid, gives a yellow coloration in milk, and liquefies starch with the formation of acid; series (*c*) causes clearing of milk, and gives a yellow colour on starch paste. The majority appear to be related to *P. chrysogenum*, Thom, and *P. citrinum*, Thom; series (*a*) agrees well with *P. candidofulvum*, Biourge, and sp. 82 is almost certainly *P. Roqueforti*.

Until the nomenclature of this genus is more firmly established, caution would seem to be more desirable than too ready identification. It is still the practice of many workers to refer to any blue-green *Penicillium* as *P. glaucum*.

Other Genera

Of the other genera *Fusarium* is probably the most important as regards colour production, a number of species causing red and purple stains on cloth.

Mucor (including *Rhizopus*) is common in raw cotton, and some species are capable of producing tendering; but they do not develop freely at the humidities usually encountered by cloth, nor do they produce stains.

Cladosporium is a common cause of dark stains, as are *Stemphylium* and several other fungi belonging to the *Dematiaceæ*.

For the identification of the *Fungi Imperfecti*, Lindau's classification[9] is very useful; a summary and translation of the part relating to soil fungi is given by Waksman.[18]

Thanks are due to Mr. G. Smith, M.Sc., for a number of species isolated from cotton yarn and cloth. In the culture work recorded in this paper, considerable assistance has been received from Mr. G. R. Bates, B.Sc.

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IV—THE INHIBITORY ACTION OF CERTAIN SUBSTANCES ON THE GROWTH OF MOULD FUNGI

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The liability of grey and, to a less extent, of finished cloths to become mildewed in a warm humid atmosphere has led to the use of antiseptics to prevent, or hinder, the growth of mould fungi. The antiseptic that has been used most widely is zinc chloride, and when present in adequate amount it has on the whole led to satisfactory results. The concentration needed to give adequate protection, however, is at least 0·8% on the total weight of the cloth, and there are many instances where this addition is either difficult or impossible, or where the use of chlorides is definitely prohibited. On this account other antiseptics have been introduced from time to time, chief amongst them sodium silicofluoride, phenol, the cresols, salicylic acid, and trichlorophenol. None of them, however, has proved a satisfactory alternative to zinc chloride.

An antiseptic suitable for general use in the cotton industry must possess most of the following properties in addition to high antiseptic efficiency.

(1) The antiseptic must not volatilise during the boiling of the size mixing or finishing paste, or the drying of the sized yarn or filled cloth.

(2) It must be sufficiently soluble to ensure uniform distribution throughout the mixing.

(3) It must be unaffected by heat, by the metallic surfaces with which it may come in contact, and by any of the ingredients commonly used in sizes and finishing pastes.

(4) The antiseptic must not have any appreciable colour or smell, and must not develop any colour at slightly acid or alkaline reactions.

(5) It must not have any action on cotton.

(6) If used in a cloth that is to be dyed or finished subsequently it must not affect these processes.

(7) To be generally useful, the cost, for equal protection, should not be greater than that of zinc chloride.

For a long time the staff of the Shirley Institute have been seeking an ideal antiseptic for use in the cotton industry to protect textile materials from fungoid attack. As little is known of any relation between chemical constitution and fungicidal properties there have been few indications to guide the search, so that substances of many types have been prepared and tested. This paper gives a summarised account of the results of the tests, and describes briefly the preparation of those substances that are either uncommon or previously unknown. The biological tests were begun by L. E. Morris and extended by one of us (L.D.G.).

In an earlier paper, Morris[27] described a method for testing the value of different substances as mildew antiseptics. This consisted in measuring the rate at which selected organisms grew on suitable sterile media containing different amounts of the substance under test. The novel feature of the method lay in the use of a number of different organisms, selected either 'because they occurred commonly in mildewed cloths or because they were

specially resistant to antiseptics. This practice has been continued in the present experiments, for it affords the simplest means of selecting the few substances that merit fuller examination. These have been incorporated in a range of size mixings, and their effects tested on cloths woven with the sized warps. The present memoir, however, deals only with the results obtained on nutrient media in the laboratory.

Morris[27] made a preliminary survey of the antiseptics that were either used or recommended for use in the cotton industry and also examined other substances which, either because they were used in other industries or on general grounds, appeared to be possible antiseptics. The survey revealed three antiseptics of exceptional toxicity—thallium carbonate, *p*-nitrophenol, and 2:4:6-trichlorophenol. The first of these was in many ways ideal, but there proved to be a serious objection to its general use, for the available supplies of thallium are very limited and the price is therefore so high as to make it much more expensive to use than zinc chloride. The second—*p*-nitrophenol—was both toxic and cheap, but the colour that developed in neutral or alkaline mixings precluded its general use. The third—trichlorophenol—was both pungent and volatile.

In general the investigation showed that the toxicity of phenol was increased by the introduction of alkyl, nitro, or halogen groups, whilst later experiments have shown that it is diminished by the introduction of acrylic acid, sulphonic acid, or additional hydroxyl groups, the effect of the sulphonic acid group more than counteracting that of the nitro-group.

A survey of the toxicity of the salts of different metals did not reveal any substance of outstanding merit; the search was therefore continued to find a suitable organic antiseptic.

In the first place, attention was directed to the possibility of minimising the undesirable characteristics of *p*-nitrophenol or the halogenated phenols. Acetylation of nitrophenol eliminates the colour, but the presence of acetyl *p*-nitrophenol in the requisite amount to prevent mould growth can still be detected by the colour imparted to the cloth at reactions more alkaline than *p*H6 (probably owing to hydrolysis), so that although acetylation reduced the toxicity only to one-half, the use of acetyl-*p*-nitrophenol was no more practicable than that of the parent substance. Conversion of nitrophenol into the more soluble and colourless α-glyceryl ether also failed, the ether being very much less toxic than *p*-nitrophenol. Similarly, acetylation of the halogenated phenols minimises their smell and at the same time reduces the toxicity to half, but acetyl trichlorophenol is at least as volatile as the parent substance. As with nitrophenol, the glyceryl ethers proved to be practically non-toxic.

The scope of later exploratory work is indicated in Table I. Arsenic, antimony, selenium, and thallium in organic combination proved ineffective, and although boron was more promising, it was not sufficiently so to warrant fuller investigation. The experiments led, however, to three lines of attack (*a*) acetanilide and its derivatives, (*b*) anilides of acids other than acetic acid, and (*c*) the organomercury compounds.

Acetanilide proved to be about half as toxic as phenol, and on this account its derivatives and other allied substances were investigated. The introduction of substituents into acetanilide, however, generally leads to a fall in toxicity, slight for methyl (*o* or *p*), chlorine (*p*), or nitro (*p*), and very marked for hydroxyl (*p*) or N-methyl. The toxicities of acet-β-naphthyl-

amide and 8-acetylaminoquinoline are similar to that of acetanilide, whilst acet-α-naphthylamide, 5-acetylaminoquinoline and 2-acetylaminopyridine are relatively non-toxic. One feature is perhaps worth noting. Acet-*o*-toluidide, acet-α-naphthylamide, and 5-acetylaminoquinoline, which contain a carbon substituent in *ortho* position to the acetylamino-group, are less toxic respectively than acet-*p*-toluidide, acet-β-naphthylamide, and 8-acetylaminoquinoline.

The combination of aniline with other acids led to only one substance of definite promise—salicylanilide—which is as toxic as thallium carbonate and meets the requirements for an ideal antiseptic enumerated elsewhere, save for its slight solubility. Direct sizing experiments have shown, however, that this does not prevent it from being distributed uniformly throughout a mixing or on a warp in the quantity necessary to afford efficient protection against mould growth. If it is required in a soluble form, the readily soluble sodium salt may be employed without detriment to its toxicity. Salicylanilide proved to be quite exceptional, and of other salicyl derivatives that have been prepared only the *o*-anisidide approaches it in toxicity. Replacement of aniline by *o*- or *p*-toluidine, phenylhydrazine, *p*-aminophenol, or *p*-chloroaniline reduces the toxicity to less than one-tenth, whilst replacement by methylaniline, *m*- or *p*-phenylenediamine, benzidine, α- or β-naphthylamine, *m*-aminobenzoic acid, or 5-aminosalicylic acid leads to products of very low toxicity. Similarly, replacement of salicylic acid by benzoic or *p*-hydroxybenzoic acids leads to diminished toxicity.

Introduction of mercury into the nucleus of phenol gives rise to two monosubstituted derivatives. The more soluble of these—tested in the form of *o*-chloromercuriphenol—is seven times as toxic towards mould fungi as mercuric chloride, but the less soluble *para* isomeride is only as toxic as mercuric chloride. A number of *o*-chloromercuriphenols with different substituents have been examined, but all of them are less toxic than the parent substance, the effect of the substituent in lowering the toxicity increasing in the order—

$$o\text{-Me} ; \rightarrow \begin{cases} p\text{-Me} \\ p\text{-Cl} \\ p\text{-isobutyl} \end{cases} \rightarrow \begin{cases} p\text{-isoamyl} \\ p\text{-bromo} \\ 2{:}4\text{-dibromo} \end{cases} \rightarrow \begin{cases} p\text{-nitro} \\ p\text{-sulphonic acid} \end{cases}$$

p-Acetoxymercuriacetanilide is almost as toxic as *o*-chloromercuriphenol; the *ortho* isomeride is only one-third as toxic as the *para* whilst *o*-acetoxymercuri-*p*-toluidide is very much less toxic.

Solutions of these mercury compounds are much more stable to metals than are the inorganic mercury salts, but in boiling solution there is sufficient interaction to render them unsuitable for use in a size mixing.

The research has therefore revealed a number of substances of outstanding toxicity towards mould fungi, chief amongst them salicylanilide, *o*-chloromercuriphenol, *p*-acetoxymercuriacetanilide, thallium carbonate, *p*-nitrophenol, and trichlorophenol. Of these only salicylanilide* appears to combine a sufficient number of the desirable properties of an antiseptic for general use in the cotton industry. In view of its possible application in other spheres, it is of interest to record that solutions of salicylanilide appear to be non-toxic to plant life.

* The protection of textile and other materials from fungal attack by the use of salicylanilide is the subject of a patent; the substance is marketed by Imperial Chemical Industries Ltd. (British Dyestuffs Corporation) under their registered name "Shirlan."

EXPERIMENTAL

Part I. Biological

Method of Testing

The method of testing has been described fully by Morris[27]; the procedure need therefore be only briefly summarised.

The tests were done in sterilised media made from soft wheat flour and containing antiseptics in definite concentrations, calculated as a percentage (of anhydrous substance) on the weight of the medium. The medium usually employed was a 6% wheat flour paste, filled into test-tubes and sterilised by steaming in an autoclave on three successive days. The tubes were inoculated in duplicate with pure cultures, and incubated at 25° C. The "inhibiting concentration" is taken as that at which none of the fungi tested showed any growth in 20 days. Sometimes for further examination a 2% flour paste stiffened with 2% of agar was used. The results obtained are substantially the same as with the other medium, but the use of flour agar enables any slight retardation of growth to be more easily detected. Petri dishes containing the solidified agar medium were inverted, inoculated (each in triplicate) at the centre with pure culture, and incubated. The rate of growth was then studied by measuring at regular intervals the diameter of the circular colony that developed. The interpretation of such measurements has already been discussed.[27]

The Organisms Employed in Testing

As a result of the examination of numerous samples of mildewed cloth, yarn, and raw cotton, a collection of 180 mould fungi has been made, and, as far as possible, classified. The collection is described in the preceding memoir, and the record numbers given in the key to species in Table I are those used in that description.

The antiseptic tests were done with a number of these fungi chosen as being (*a*) types commonly occurring in cotton goods, and (*b*) the most generally resistant of such types to antiseptics. In most of the earlier tests the following species were used—*Penicillium* spp. 2 and 4; *Aspergillus flavus* 35; *A. versicolor* 45; *A. niger* 8; *Rhizopus arrhizus* 34; *Cladosporium herbarum* 15; and *Fusarium* sp. 36. Later, this selection was changed to the following as being more representative—*Aspergillus flavus* 35; *A. versicolor* 45; *A. terreus* 48; *A. fumigatus* 74; *Penicillium* sp. 85, and *Cladosporium herbarum* 15.

In addition, other common types have been substituted from time to time, and with some antiseptics a more extended range has been tried. The different mildew fungi show considerable specificity in their behaviour to antiseptics. In general, *Aspergillus flavus* 35 is the most hardy of the species tried. With organic mercury compounds, however, *Penicillium* 85 and 86 withstand concentrations several times greater than those given as the "inhibiting concentrations" in Table I. With thallium compounds *Cladosporium* is the most resistant species, and similar variations are shown with some of the other antiseptics. None of the fungi tested has proved unusually resistant to salicylanilide.

Aspergillus glaucus spp., when introduced into a test (see, for example, mercurous chloride, N-methylacetanilide, chlorinated tar phenols) have been the first suppressed.

Summary of Results

A list of the substances tested is given in Table I, together with an indication of the effect of certain concentrations on mildew fungi.

The list includes some 60 results, published and unpublished, obtained by Morris.

The inhibiting concentration has not always been determined, since the highest concentrations tested have sometimes been limited by practical difficulties, such as the slight solubility of the substance under the conditions of the test, and sometimes by technical considerations such as the cost of the substance. Three columns are therefore given, showing the concentrations corresponding to no growth, slight growth, and unrestricted growth respectively. For the sake of convenience, the fungi have been represented by letters, these and the record numbers being given in the key to species at the head of the table.

Bactericidal Action

The bactericidal properties of the substances have not been exhaustively tested. It has been observed, however, that fungicidal power and bactericidal power are not necessarily concomitant; salicylanilide and thallium carbonate, for example, are poor bactericides.

Of the less well-known substances in Table I, several are worthy of attention as possible bactericides. For example, sodium silicofluoride inhibited the development of a mixed inoculation of soil bacteria in broth in a concentration of 0·05 per cent. Diphenyliodonium chloride under similar conditions inhibited development in a concentration of 0·01% and appears to be comparable in bactericidal power with mercuric chloride.

Table I
Biological Tests on Various Antiseptics

NOTES

The most resistant species are indicated by reference letters in bold type.

Letters in brackets indicate species that are resistant to concentrations higher than those quoted as inhibiting growth.

The reference numbers are those assigned to the cultures maintained at the Shirley Institute.

* More effective the more acid the medium.

† More effective the more alkaline the medium.

‡ 0·015% has a considerable effect.

Substances described in this paper are marked thus (§). Others were made in the laboratory and a reference is given to the literature describing the method of preparation of the less well-known of them. A few rare substances were provided by other workers. The remainder were purchased from chemical manufacturers and purified when necessary.

KEY TO MOULD FUNGI EMPLOYED IN THE TESTS

	Letter	Reference No.		Letter	Reference No.
Penicillium spp.	a	2	*A. versicolor* sp.	o	60
	b	4	*A. Sydowi* sp.	p	61
	c	42	*Aspergillus fumigatus* ...	q	74
	d	68	*Aspergillus Wentii*	r	75
	e	85	*A. ruber* sp.	s	23
	f	86	*Aspergillus repens*	t	26
Aspergillus niger	g	8	*Cladosporium herbarum* ...	u	15
Aspergillus flavus	h	35	*Fusarium* spp.	v	32
A. Sydowi sp.	j	44		w	36
A. versicolor spp.	k	45	*Rhizopus arrhizus*	x	34
	l	46	*Rhizopus nigricans*	y	84
Aspergillus terreus	m	48	*Alternaria tenuis*	z	37
Aspergillus ruber sp. ...	n	55			

Table I—Biological Tests on Various Antiseptics—*cont.*

No.	Substance	% Concentration of Antiseptic: Growth inhibited	Effect marked	Effect slight or absent	Species tested
	Inorganic Compounds				
1	Alum (potassium)	—	0·5	—	e, **h**, k, m, **q**, u
2	Aluminium fluoride ...	—	0·2	—	a, b, g, h, k, l, u, w
3	Aluminium sulphate ...	—	0·36	—	e, **h**, k, m, **q**, u
4	Ammonium fluoride ...	0·04	—	—	a, b, g, h, k, l, u, w, x
5	Barium nitrate	—	0·5	—	a, b, g, h, k, l, u, w
6	Borax	0·9	—	—	a, b, g, h, k, l, u, w
7	Boric acid	—	3·0	—	a, b, c, g, h, k, u, w, x
8	Cadmium bromide‡ ...	—	0·09	—	a, **b**, g, h, **k**, l, u, w
9	Cadmium chloride‡ ...	—	0·09	—	a, **b**, g, h, **k**, l, u, w
10	Cadmium sulphate ...	—	0·09	—	a, b, g, h, k, l, u, w
11	Cerous nitrate	—	0·09	—	a, b, g, h, k, l, u, w
12	Copper chloride	—	0·1	—	a, b, g, h, k, l, u, w
13	Copper sulphate	—	0·1	—	a, b, g, h, k, l, u, w
14	Lanthanum nitrate ...	—	0·09	—	a, b, g, h, k, l, u, w
15	Lead nitrate	—	0·1	—	a, b, g, h, k, l, u, w
16	Magnesium silicofluoride ...	0·025	—	—	a, b, g, h, k, l, u, w
17	Mercuric chloride	0·02	—	—	a, b, (e), g, h, k, l, m, g, t, u, w
18	Mercurous chloride ...	—	0·03	—	a, h, k, m, q, s
19	Rubidium carbonate ...	—	1·0	—	a, b, g, h, k, l, u, w
20	Rubidium chloride ...	—	1·0	—	a, b, g, h, k, l, u, w
21	Sodium fluoride	0·8	—	—	a, g, h, u, w, x
22	Sodium silicofluoride ...	0·15	—	—	**a**, b, g, h, k, u, w, x
23	Thallium carbonate† ...	0·02	—	—	a, b, d, f, g, h, j, k, l, m, p, **u**, v, w, x, z
24	Thallium nitrate	0·02	—	—	b, h, k, u
25	Thallium sulphate	0·02	—	—	b, h, k, u
26	Thorium nitrate	—	0·09	—	e, b, g, h, k, l, u, w
27	Uranium nitrate	0·1–0·5	—	—	a, h, k, m, u
28	Zinc ammonium fluoride ...	0·1	—	—	**a**, b, g, h, u, v, x, z
29	Zinc chloride	0·8	—	—	a, b, c, d, g, h, k, m, p, u, **v**, **w**, x, z
30	Zinc hydroxide	1·0	—	—	a, b, g, h, u, v, x, z
31	Zinc silicofluoride	—	0·13	—	a, g, h
32	Zinc sulphate	1·0–2·0	—	—	a, b, g, h, k, u, v, w, x
	Organo-mercury Compounds				
33	*o*-Acetoxymercuriacetanilide (§)	—	0·01	·005	e, g, h, m, q, u
34	*p*-Acetoxymercuriacetanilide (§)	0·005	—	—	d, (e), g, h, k, m, q, u
35	2-Acetoxymercuriacet-*p*-toluidide[35]	—	—	0·01	e, h, k, m, q, u
36	Chloromercuri-*p*-bromophenol (§)	—	0·01	—	(e), h, k, m, q, u
37	Chloromercuricarvacrol[18] ...	—	—	0·02	(e), h, k, m, q, u
38	Chloromercuri-*o*-chlorophenol (§)	0·01	—	—	(e), h, k, m, q, u
39	Chloromercuri-*p*-chlorophenol (§)	0·01	—	—	(e), h, k, q, r, u
40	Chloromercuri-*o*-cresol (m.p. 183° C.) (§) ...	0·005–0·01	—	—	(e), h, k, m, q, r, u
41	Chloromercuri-*o*-cresol (m.p. 210° C.) (§) ...	0·005–0·01	—	—	(e), h, k, q, r, u
42	2-Chloromercuri-*p*-cresol*[13]	0·01	—	—	d, (e), g, h, k, m, q, u

Table I—Biological Tests on Various Antiseptics—*cont.*

No.	Substance	% Concentration of Antiseptic			Species tested
		Growth inhibited	Effect marked	Effect slight or absent	
Organo-mercury Compounds—*cont.*					
43	Chloromercuri-2:4-dibromophenol (§)	—	0·01	—	(e), h, k, q, r, u
44	2-Chloromercuri-*p*-isoamylphenol[18]	—	0·01	—	(e), h, k, q, r, u
45	2-Chloromercuri-*p*-nitrophenol (§)	—	—	0·01	(e), h, k, m, q, u
46	*o*-Chloromercuriphenol (§)	0·003	—	—	a, d, (e), (f), g, h, l, m, n, q, r, t, u, w, y
47	*p*-Chloromercuriphenol (§)	0·01–0·02	—	—	d, (e), g, h, k, m, q, u
48	2-Chloromercuri-*p*-tert.-butylphenol[19]	0·02	—	—	(e), h, k, m, q, u
49	Dichloromercuri-*p*-chlorophenol (§)	—	0·01	—	(e), h, k, m, q, u
50	Dichloromercuriphenol-*p*-sulphonic acid (§)	—	—	0·01	(e), h, k, m, q, u
51	Chloromercuri derivatives of chlorinated low temp. tar phenols, b.p. 75–130°/3 mm. (§)	0·02	—	—	(e), h, k, m, q, u
52	Chloromercuri derivatives of low temp. tar phenols, b.p. 185–190° C. (§)	0·01–0·02	—	—	(e), h, k, m, q, u
53	Chloromercuri derivatives of low temp. tar phenols, b.p. 195–200° C. (§)	—	0·02	—	(e), h, k, m, q, u
Organic Compounds					
The substances are arranged in alphabetical order, but terms used to indicate the position of substituent groups are not considered in the alphabetical arrangement. Metallic salts of acids and phenols are given under the acid or phenol.					
54	2-Acetylaminopyridine[7,39]	—	—	0·1–0·3	e, h, k, m, q, u
55	5-Acetylaminoquinoline[8]	—	—	0·3	e, h, k, m, q, u
56	8-Acetylaminoquinoline[8]	0·3	0·1	—	e, h, k, m, q, u
57	Acetanilide	0·3	—	—	a, **h**, k, l, m, q, u, w
58	Acet-*α*-naphthylamide	—	—	0·3	e, h, k, m, q, u
59	Acet-*β*-naphthylamide	—	0·05–0·2	—	e, h, k, m, q, u
60	Acet-*o*-toluidide	—	0·3	—	e, h, k, m, q, u
61	Acet-*p*-toluidide	0·3	—	—	e, **h**, k, m, q, u
62	*p*-Acetylaminophenylstibinic acid, sodium salt[14]	—	—	0·02	e, g, h, k, q, u
63	Acetyl-*p*-nitrophenol*	0·02–0·03	—	—	a, b, g, h, k, l, u, w
64	Acetylsalicylic acid	0·1	0·05	—	e, h, k, m, q, u
65	Acetyltribromophenol[34]	0·01–0·02	—	—	e, g, h, k, q, u
66	*p*-Aminophenylarsinic acid	—	—	0·02	e, g, h, k, q, u
67	5-Aminoquinoline[8]	0·3	—	0·1	e, h, k, m, q, u
68	Benzanilide	—	—	0·1	e, h, k, m, q, u
69	Benzoic acid	0·05	—	—	a, b, g, h, k, l, o, p, u, w
70	Benzylacetamide[1]	—	—	0·1	e, h, k, m, q, u
71	*o*-Bromophenol	0·07	—	—	a, b, g, h, v, x
72	Caprylanilide	—	—	0·3	e, h, k, m, q, u
73	*p*-Chloroacetanilide	—	0·3	—	a, h, l, m, t, u
74	Chlorinated low temp. tar phenols, b.p. 120–165°/3 mm.	—	0·1	—	a, g, h, k, m, q, s, t

Table I—Biological Tests on Various Antiseptics—*cont.*

No.	Substance	% Concentration of Antiseptic: Growth inhibited	Effect marked	Effect slight or absent	Species tested
	Organic Compounds—*cont.*				
75	4-Chloro-*m*-cresol	0·05	—	—	a, b, g, h, k, l, u, w
76	*o*-Chlorophenol	0·08	—	—	a, b, g, h, v, x
77	Chlorothymol	—	0·05	—	a, b, g, h, k, l, u, w
78	Cinnamanilide	—	—	0·3	e, h, k, m, q, u
79	Cinnamic acid	—	0·1	0·05	e, h, k, m, q, u
80	Cresol (mixed)	0·1	—	—	a, b, g, h, u, w
81	*m*-Cresol	0·1	—	—	a, g, h
82	*p*-Cresotinanilide[4]	—	—	0·1	e, h, k, m, q, u
83	Diacet-*m*-phenylene-diamide[5]	—	—	0·3	e, h, k, m, q, u
84	2:4 Dibromophenol	0·01	—	—	a, b, g, h, u, v, x, z
85	Dichloro-*o*-cresol	0·05	—	—	a, g, h, x
86	2:4-Dinitrophenol	0·02	—	—	a, b, g, h, k, l, u, v, w, x, z
87	Diphenyliodonium chloride[40]	—	0·2	—	a, h, k, l, u, w
88	Disalicylbenzidide (§)	—	—	0·1	e, h, k, m, q, u
89	Disalicyl-*m*-phenylene-diamide (§)	—	—	0·1	e, h, k, m, q, u
90	Disalicyl-*p*-phenylene-diamide (§)	—	—	0·1	e, h, k, m, q, u
91	Formaldehyde	0·05	—	—	a, b, g, h, u, v, x, z
92	Glyceryl-*p*-nitrophenol[6]	—	—	0·1	e, g, h, k, q, u
93	Glyceryl tribromophenol[24]	—	—	0·03	d, g, h, m, q, w
94	Hexamine	—	0·5	—	a, b, g, h, k, u, v, x
95	Hexylresorcinol	—	0·05	—	e, h, k, m, q, u
96	Hydrocinnamanilide	—	—	0·3	e, h, k, m, q, u
97	*p*-Hydroxyacetanilide	—	—	0·5	e, g, h, k, q, u
98	*p*-Hydroxybenzanilide	—	—	0·1	e, h, k, m, q, u
99	*p*-Hydroxybenzoic acid	—	0·04	—	a, b, g, h, k, l, u, w
100	*o*-Hydroxycinnamic acid (coumaric acid)	—	0·3	0·1	e, h, k, m, q, u
101	*p*-Hydroxycinnamic acid[31]	0·3	0·1	—	e, h, k, m, q, u
102	*p*-Hydroxyphenylacetic acid	—	—	0·3–0·1	e, h, k, m, q, u
103	*p*-Hydroxyphenylarsinic acid	—	—	0·02	e, g, h, k, q, u
104	N-Methylacetanilide	—	—	0·3	a, h, k, m, q, s
105	Monoacetyl-*p*-phenylene-diamine[28]	—	—	0·3	e, h, k, m, q, u
106	Naphthalene	0·25	—	—	a, b, g, h, u, v, x, z
107	Naphthalene sulphonic acids and sodium salts	—	—	0·25	a, b, g, h, k, u, v, x
108	*p*-Nitroacetanilide	—	0·4	—	a, h, k, l, u, w
109	3-Nitro-*p*-hydroxybenzoic acid	0·05–0·1	—	—	a, b, g, h, k, l, u,w
110	*p*-Nitrophenol*	0·017	—	—	a, **b**, **c**, g, h, k, l, m, u, v, w
111	*p*-Nitrophenol-2-sulphonic acid	—	0·03	—	a, b, g, h, k, l, u, w
112	5-Nitrosalicylic acid	—	0·1	—	a, b, g, h, k, l, u, w
113	Oxanilic acid	—	—	0·1	e, h, k, m, q, u
114	Oxanilic acid, sodium salt	—	—	0·3	e, h, k, m, q, u
115	Phenol	0·13	—	—	a, b, g, **h**, k, l, q, t, u, v, w, x, z
116	Phenol, sodium salt	0·2	—	—	a, b, g, h, k, l, u, w
117	Phenol-*o*-sulphonic acid	—	0·3	—	a, b, g, h, k, l, u, w
118	Phenol-*p*-sulphonic acid	—	0·3	—	**a**, b, **g**, h, k, l, **u**, w
119	Phenol-*p*-sulphonic acid magnesium salt	—	0·3	—	a, b, g, h, k, l, u, w

Table I—Biological Tests on Various Antiseptics—*cont.*

No.	Substance	% Concentration of Antiseptic: Growth inhibited	Effect marked	Effect slight or absent	Species tested
	Organic Compounds—*cont.*				
120	Phenolsulphonic acid, mixed, copper salt ...	0·1	0·05	—	e, h, k, m, q, u
121	Phenolsulphonic acid, mixed, zinc salt ...	3·0	—	—	a, b, h, v
122	Phenylacetic acid	0·3	0·02	—	e, h, k, m, q, u
123	Phenylboric acid[25] ...	0·06–0·1	—	—	b, e, **g**, h, k, u
124	Phenylboric acid, sodium salt	0·06–0·1	—	—	b, e, g, h, k, u
125	2-Phenylglyoxaline[15] ...	0·3	0·1	—	e, h, k, m, q, u
126	Phenylpropionic acid (hydrocinnamic acid) ...	0·2	0·1	—	e, h, k, m, q, u
127	Phenyl salicylate (salol) ...	—	0·08	—	a, b, g, h, k, u, w, x
128	Phenylthiourethane ...	—	0·05	—	e, h, k, m, q, u
129	Phenylurea	—	—	0·4	a, h, l, m, t, u
130	Resorcinol	—	—	0·1	a, h, k, m, q, s
131	Saccharin	—	—	0·01–0·1	e, h, k, m, q, u
132	Salicylamide	—	0·1	0·05	e, h, k, m, q, u
133	Salicylyl-*m*-aminobenzoic acid (§)	—	—	0·1	e, h, k, m, q, u
134	N-Salicylyl-*p*-aminophenol	(§) —	0·2	0·1	e, h, k, m, q, u
135	N-Salicylyl-5-aminosalicylic acid (§)	—	—	0·1	e, h, k, m, q, u
136	Salicylanilide ("Shirlan") (§)	—	0·005	—	e, **h**, k, m, q, u
	A 0·01% solution suppresses all but the most resistant species. Owing to the slight solubility of Shirlan, higher concentrations cannot conveniently be tested.				
137	Salicylanilide, sodium salt ("Shirlan S.") (§) ...	0·02	0·005	—	e, g, h, k, m, n, q, u, w, y
138	Salicyl-*o*-anisidide (§) ...	—	0·03	0·02	e, h, k, m, q, u
139	Salicyl-*p*-chloroanilide (§)	—	0·1	0·02	e, h, k, m, q, u
140	N-Salicyl-*β*-hydroxyethyl-anilide (§)	—	—	0·1	e, h, k, m, q, u
141	Salicylic acid	0·05	—	—	a, b, c, g, h, k, u, w, x
142	Salicylic acid, zinc salt ...	—	0·08	—	a, g, h, x
143	Salicyl-N-methylanilide (§)	—	—	0·1	e, h, k, m, q, u
144	Salicyl-*α*-naphthylamide (§)	—	—	0·1	e, h, k, m, q, u
145	Salicyl-*β*-naphthylamide (§)	—	—	0·1	e, h, k, m, q, u
146	Salicylphenylhydrazide[9] ...	—	0·02–0·1	—	e, h, k, m, q, u
147	Salicyl-*o*-toluidide[30] ...	—	0·1	0·02	e, h, k, m, q, u
148	Salicyl-*p*-toluidide[2,36] ...	—	0·1	0·02	e, h, k, m, q, u
149	Sulphosalicylic acid ...	—	—	0·1	e, h, k, m, q, u
150	Tetrabromocresol	0·03	—	—	a, b, g, h, u, v, x, z
151	Tetrahydronaphthalene ...	0·3	—	—	a, b, g, h, u, v, x, z
152	Thallium dimethyl iodide[16]	—	—	0·05	e, h, k, m, q, u
153	Thiocarbamide	0·8	—	—	a, g, h, x
154	Thymol	0·04	—	—	a, b, g, h, k, l, u, w
155	*p*-Toluenesulphonanilide ...	—	—	0·1	e, h, k, m, q, u
156	*p*-Toluenesulphonic acid, zinc salt	—	0·5	—	e, h, k, m, q, u
157	Tribromophenol	0·005–0·01	—	—	a, b, e, g, h, k, l, q, u, v, x, z
158	Tribromophenol, sodium salt	0·007	—	—	a, b, g, h, k, l, u, w
159	Trichlorophenol	0·005–0·01	—	—	a, b, g, h, k, l, u, **w**
160	Trichlorophenol, sodium salt	0·007	—	—	a, b, g, h, k, l, u, w
161	Trihydroxytriphenyl selenonium nitrate[26] ...	—	—	0·05	e, h, k, m, q, u

Part II. Chemical

Preparation of Salicyl Derivatives by Means of Salicyl Chloride

Salicyl chloride was prepared as described by Kopetschni and Karczag,[21] save that the thionyl chloride was diluted with benzene prior to mixing with sodium salicylate. Thionyl chloride (1 mol.) was mixed with twice its volume of dry benzene in a short-necked flask and treated gradually with dry sodium salicylate (1 mol.) with constant shaking and cooling. The flask was then closed with a calcium chloride drying tube and kept at room temperature overnight. The gelatinous product was thoroughly mixed with dry benzene and slowly added to a well-cooled solution or suspension of the appropriate base (2 equivalents) in dry benzene. After standing for a short time, the mixture was diluted with ether, shaken with dilute hydrochloric acid to remove unused base, washed with water, dried with anhydrous sodium sulphate, and the solvent then removed. The residue was dissolved in sodium hydroxide solution, decolorised by means of charcoal, and partly neutralised, after which carbon dioxide was passed into the solution until it was no longer alkaline to phenol red. The precipitated salicyl derivative, separated in this way from salicylic acid, was collected, washed with water, and dried.

The yield of *salicylanilide* obtained by this method was 80% of the theoretical, calculated on the sodium salicylate used; this corresponds with the yields of salicyl chloride obtained by other workers (cf. Kopetschni and Karczag,[21] and Anschütz and Riepenkroger[3]).

The interaction of the aluminium derivative of aniline with salicylic acid according to German Patent 347,607 was also investigated; the yield of salicylanilide never exceeded 9 per cent of the theoretical.

Salicylanilide dissolves very sparingly in water, 100 g. dissolving 0·005 g. at 25° C. and 0·08 g. at 100° C. It does not give any coloration with ferric chloride in aqueous solution, but alcoholic ferric chloride produces a violet colour which fades very slowly if the alcohol is free from water, but rapidly if it contains more than 2% of water.

The *sodium* salt crystallises from water with $4H_2O$. (Found, loss at 110° C., 23·4%; $C_{13}H_{10}O_2NNa$, $4H_2O$ requires 23·5 per cent.) One hundred grams of water at 25° C. dissolve 31·7 g. of the hydrated salt. The salt is stable, for after exposure in a thin layer for a month 94% was recovered unchanged. The *calcium and barium* salts are both soluble in water.

Salicyl-α-naphthylamide[33] was obtained in 51% yield.

Salicyl-β-naphthylamide was obtained in 45% yield; melting point 187° C.; Senier and Shepheard[33] and Loevenitch and Loeser[22] give 188–189° C.

Salicyl-p-*chloroanilide* crystallises from 80% alcohol in flattened needles melting at 168–169° C. Yield, 57%; 100 g. of water dissolve 0·004 g. at 25° C. (Found—Cl, 14·3; $C_{13}H_{10}O_2NCl$ requires : Cl, 14·3 per cent.)

N-methylsalicylanilide, prepared from monomethylaniline, separates from light petroleum in clusters of stout rhombic prisms melting at 113–114° C. Yield, 72 per cent. (Found—N, 6·3; $C_{14}H_{13}O_2N$ requires : N, 6·2 per cent.) It is readily soluble in ether, benzene, or ethyl acetate, and fairly readily soluble in alcohol; 100 g. of water dissolve 0·019 g. at 25° C. Unlike N-methylacetanilide[10] it is resistant to hydrolysis by water, 85% being recovered unaltered after boiling for 4 hours.

Salicyl-o-*anisidide* crystallises from light petroleum in large concentric masses of needles melting at 115° C. Yield, 78 per cent. (Found—N, 5·9; $C_{14}H_{13}O_3N$ requires : N, 5·8 per cent.)

Salicyl-N-β-hydroxyethylanilide was obtained in 63% yield from β-hydroxyethylaniline. It separates from a mixture of benzene and light petroleum in glistening flattened needles melting at 100° C. It dissolves readily in ether, benzene, acetone, chloroform or ethyl acetate, but only sparingly in light petroleum. (Found—N, 5·6; $C_{15}H_{15}O_3N$ requires : N, 5·45 per cent.)

Salicylyl-5-aminosalicylic acid. No interaction appeared to take place between a suspension of the amino-acid in benzene and salicyl chloride in the cold. The mixture was therefore boiled gently for 4 hours, moisture being carefully excluded. When cold, the solid product of the reaction was washed with dilute hydrochloric acid to remove unchanged amino-acid, dried, and finally washed well with ether. Yield, 20 per cent. The substance blackens slowly when heated and decomposes at about 270° C.; it dissolved so sparingly in all the usual solvents that all attempts at crystallisation were unsuccessful. (Found—N, 5·1; $C_{14}H_{11}O_5N$ requires : N, 5·1 per cent.)

Salicyl Derivatives Prepared by Means of Salol

This method was first described by Cohn[9] and gives almost a theoretical yield of salicylanilide.

Salicylyl-p-*aminophenol* (*p*-hydroxysalicylanilide) was prepared by heating a mixture of salol (1 mol.) and *p*-aminophenol (1·1 mol.) dissolved in phenol in an oil bath at 200° C. for 4 hours. After crystallisation first from aqueous alcohol and then from boiling water it melted at 176° C. (Cohn[9] gives 168–169°). (Found—N, 6·2; $C_{13}H_{11}O_3N$ requires : N, 6·1 per cent.)

Salicylyl-m-*aminobenzoic acid.* *m*-Aminobenzoic was dissolved in molten salol and heated at 180° C. for 1 hour, during which time the product gradually crystallised. When cold, the mass was broken up and washed thoroughly with ether. Yield, 85 per cent. It is extremely insoluble in all the usual solvents with the exception of acetone, from which it separates very slowly in microcrystalline nodules decomposing at 275° C. (Found—N, 5·7; $C_{14}H_{11}O_4N$ requires : N, 5·45 per cent.)

Disalicyl-m-*phenylenediamide.* Freshly distilled *m*-phenylenediamine (1 mol.) was heated with salol (2 mols.) at 185° C. for 2½ hours in an atmosphere of hydrogen. The ethereal solution of the product was washed with dilute hydrochloric acid and with water, dried, and the ether evaporated. The residue separated from alcohol in concentric clusters of stout prisms melting at 200° C. (Found—N, 8·1; $C_{20}H_{16}O_4N_2$ requires : N, 8·05 per cent.) It dissolves sparingly in ether, fairly readily in alcohol or ethyl acetate, and very readily in acetone; it is almost insoluble in benzene, light petroleum, or chloroform.

Disalicyl-p-*phenylenediamide* was prepared in the same way as the *meta* isomeride. The crude solid product was purified by washing thoroughly with ether. It is almost insoluble in the usual solvents but dissolves in hot pyridine, from which it separates in glistening needles containing solvent of crystallisation, which is rapidly lost on standing in the air or in a vacuum. (Found—N, 8·25; $C_{20}H_{16}O_4N_2$ requires : N, 8·05 per cent.)

Disalicylbenzidide was prepared in the same way as disalicyl-*p*-phenylenediamide. It is extremely insoluble in most solvents, but separates from pyridine in small glistening leaflets, which change to a powder in contact with the air or in a vacuum. (Found—N, 6·6; $C_{26}H_{20}O_4N_2$ requires : N, 6·6 per cent.) The preparation is briefly described by Cohn[9], but no analyses are recorded.

Mercury Derivatives of Phenols

The mono-mercuration of phenol. By the interaction of phenol and mercuric oxide in equimolecular proportions in acetic acid, Dimroth[12] obtained a mixture of products consisting principally of dimercury compounds of phenol, *o*-acetoxymercuriphenol accounting for only 15% of the mercuric oxide and *p*-acetoxymercuriphenol for 6 per cent. More recently, Whitmore and Middleton[38] and Mameli[23] have stated that the *gradual* addition of mercuric acetate (1 mol.) to phenol (1·5 mol.) heated on a boiling water-bath leads essentially to monomercurated derivatives. The former writers also state that the relative proportions of the two isomerides at different temperatures are as follows—

Temperature	*Ortho*	*Para*
100° C.	28%	72%
125° C.	33%	66%
150° C.	36%	64%

Mameli states that at 90–100° C. 82% of the mercuric acetate is converted into mono-mercury compounds, the relative proportions of *ortho* and *para* being 37% and 63 per cent.

Using these conditions, only 18% of the mercury was obtained in the form of mono-mercury compounds, the proportion of *ortho* to *para* being 43 to 57. Further investigation showed that the maximum yield of mono-mercurated phenols was obtained by adding the finely powdered mercuric acetate *in one operation* to the heated phenol. In this way, the following results were obtained—

Temp.	Mols. Phenol to 1 mol. $HgAc_2$	Total Yield of Mono-mercurated Phenols on $HgAc_2$	Relative Proportions	
			ortho	*para*
90° C.	1·5	71%	43	57
90° C.	2	82%	42	58
60° C.	3	88%	45	55
90° C.	3	88%	54	46
120° C.	3	83%	66	34
150° C.	3	76%	81	19

Mercuration of p-*cresol.* Mercuration of *p*-cresol (1·5 or 3 mols.) at temperatures of 60° C. to 120° C. with mercuric acetate (1 mol.) led to practically the same result throughout, 25% of the mercury being isolated as *o*-chloromercuri-*p*-cresol, and 70% as 2:6-*dichloromercuri*-p-*cresol.* This is practically insoluble in water, alcohol, acetone, benzene, or chloroform, and very sparingly soluble in boiling tetrahydronaphthalene or cyclohexanol, from which it separates in clusters of minute needles, which gradually decompose without melting above 210° C.; 100 g. water dissolve 0·001 g. at 25° C. (Found—Hg, 65·0; $C_7H_6OCl_2Hg_2$ requires : Hg, 69·3 per cent.)

Mercuration of m-*cresol.* *m*-Cresol was mercurated in the same way as phenol. The mono-mercury derivatives were isolated by dissolving the reaction product in water containing a little acetic acid, adding sodium chloride, and then cooling to 50° C., filtering off the small amount of solid chlorides that separated, and then cooling rapidly and collecting the chlorides that finally separated as a mixture of crystals and oil. This material was fractionally crystallised from benzene, yielding in the more insoluble fractions a *monochloromercuri*-m-*cresol* as radiating clusters of long glistening needles, which, when heated slowly, decomposed about 230° C. without melting.

The portion more readily soluble in benzene was purified by crystallisation from chloroform, in which the solubility of the two isomerides is reversed, yielding long needles that gradually changed to a sandy powder when left in contact with the solvent. When slowly heated they softened a little at 130–140° C. and decomposed above 230° C. without melting.

Mercuration of p-*chlorophenol.* Redistilled *p*-chlorophenol was mercurated in the same way as phenol. The product was dissolved in boiling dilute acetic acid and treated with an excess of sodium chloride. The chlorides that separated were collected, dried, and separated into two fractions by means of cold acetone, which dissolved 80% of the material. The portion soluble in cold acetone, *monochloromercuri*-p-*chlorophenol*, separated from aqueous alcohol as a mass of long curved needles or as compact aggregates of short needles according to the rate of cooling and the concentration of the solution. Both forms melted at 202–203° C. It is readily soluble in acetone and in alcohol, and fairly soluble in hot water; 100 g. of water dissolve 0·032 g. at 25° C. (Found—Hg, 55·3; $C_6H_4OCl_2Hg$ requires : Hg, 55·1 per cent.) The portion that did not dissolve in cold acetone, *dichloromercuri*-p-*chlorophenol*, separated from ethyl acetate in glistening leaflets or needles melting at 245–248° C.; 100 g. water dissolved 0·001 g. at 25° C. It was decomposed with difficulty by permanganate. (Found—Hg, 62·7; $C_6H_3OCl_3Hg_2$ requires : Hg, 67·0 per cent.)

Mercuration of p-*bromophenol.* The mixed chlorides were prepared and separated as described for *p*-chlorophenol; 70% of the products dissolved in cold acetone, the remainder being practically insoluble even in the boiling solvent.

Monochlormercuri-p-*bromophenol.* The fraction soluble in acetone was crystallised repeatedly from aqueous alcohol, from which it separates in feathery clusters of short needles, melting and decomposing at 220° C. It is readily soluble in acetone, ether, or ethyl acetate, fairly soluble in alcohol, slightly soluble in boiling water, and almost insoluble in benzene, chloroform, or light petroleum; 100 g. of water dissolve 0·012 g. at 25° C. (Found—Hg, 48·7; $C_6H_4OClBrHg$ requires : Hg, 49·1 per cent.)

Dichloromercuri-p-*bromophenol* was isolated by crystallising the fraction insoluble in acetone from ethyl acetate, from which it separates in small needles decomposing at 275–282° C. according to the rate of heating; 100 g. of water at 25° C. dissolve 0·002 g. (Found—Hg, 59·8; $C_6H_3OCl_2BrHg_2$ requires : Hg, 62·3 per cent.)

Mercuration of 2:4-*dibromophenol.* Mercuric acetate (1 mol.) dissolved in 50% alcohol containing a little acetic acid was added to 2:4 dibromophenol (1 mol.) dissolved in the same solvent. The yellow precipitate immediately formed became almost white after heating on the steam bath for 12 hours. It consists of *acetoxymercuri*-2:4-*dibromophenol*, which separates from 75% acetic acid in radiating clusters of long needles that gradually decompose above 260° C. (Found—Hg, 39·0; $C_8H_6O_3Br_2Hg$ requires : Hg, 39·2 per cent.)

Chloromercuri-2:4-*dibromophenol* is insoluble in cold and only slightly soluble in boiling water, but fairly soluble in alcohol or hot benzene, from which it separates in large aggregates of very fine needles, that melt at 240° C. (Found—Hg, 41·3; $C_6H_3OClBr_2Hg$ requires : Hg, 41·1 per cent.)

Mercuration of p-*nitrophenol.* The process used was that described by Hodgson[20] for the mercuration of *o*-nitrophenol, with the exception that two

molecules of the phenol were used instead of a large excess. The unchanged *p*-nitrophenol was removed by thorough washing with dilute acetic acid, and the residue was crystallised from 90% acetic acid. (Found—Hg, 50·6; $C_8H_7O_5NHg$ requires : Hg, 50·4 per cent.) The monoacetoxymercuri-*p*-nitrophenol was converted into the sodium salt of hydroxymercuri-*p*-nitrophenol, which was crystallised from hot water containing a slight excess of sodium hydroxide. (Found, in air-dry material—Hg, 48·4; loss at 110° C., 8·3; $C_6H_4O_4NNaHg,2H_2O$ requires : Hg, 48·4; H_2O, 8·7 per cent.) Hantzsch and Auld[17] state that the sodium salt crystallises with $\frac{1}{2}$ H_2O. Monochloromercuri-*p*-nitrophenol was prepared from the above sodium salt by treating the warm solution with dilute hydrochloric acid. The precipitated chloride was crystallised from boiling water containing a little hydrochloric acid, and formed a voluminous mass of almost colourless needles, which darken and decompose indefinitely at about 245° C. (Found—Hg, 52·4; $C_6H_4O_3NClHg$ requires : Hg, 53·6 per cent.) Hantzsch and Auld[17] give the melting point of *o*-chloromercuri-*p*-nitrophenol as 175° C., whilst Raiziss and Proskouriakoff[32] do not record a melting point. One hundred grams of water dissolve 0·018 g. at 25° C.

Mercuration of magnesium phenol-p-*sulphonate.* One molecular proportion of the salt $[(OH{\cdot}C_6H_4{\cdot}SO_3)_2Mg,8H_2O]$ was mercurated by slightly less than two molecular proportions of mercuric acetate in boiling alcohol containing a little acetic acid. A yellow precipitate began to form almost immediately. After boiling for about an hour it became white, and the solution gave no test for mercury ions. The solid was collected, ground in a mortar with a little concentrated hydrochloric acid, diluted with water and filtered. *Dichloromercuriphenol*-p-*sulphonic acid* so obtained formed a mass of minute leaflets, which separated from boiling water in fine glistening needles. (Found—Hg, 53·9; loss at 110° C., 9·7%; $C_6H_4O_4Cl_2SHg_2,4H_2O$ requires : Hg, 56·0; H_2O, 10·1 per cent.)

Mercuration of o-*chlorophenol.* The mercuration and conversion of the products into chlorides was carried out as for *p*-chlorophenol. These proved to dissolve almost completely in cold acetone; fractional crystallisation from benzene and from alcohol revealed the presence of only one *chloromercuri*-o-*chlorophenol.* This dissolved readily in acetone or ethyl acetate, fairly readily in alcohol or hot benzene, and sparingly in boiling light petroleum or chloroform. It separated from alcohol as a mass of fine needles, which melted at 185–186° C. without decomposition; 100 g. of water dissolved 0·01 g. at 25° C. (Found—Hg, 55·6; $C_6H_4OCl_2Hg$ requires : Hg, 55·2 per cent.)

Mercuration of o-*cresol.* The *o*-cresol (3 mols.) was heated at 95° C. and finely powdered mercuric acetate (1 mol.) was added with stirring; the heating was continued for 30 minutes, the product was dissolved in boiling 10% acetic acid, and the mixed mercurichlorides were precipitated by means of sodium chloride. Partial separation was effected by means of warm alcohol, which dissolved the greater part; the residue, probably *dichloromercuri*-o-*cresol,* was insoluble in the usual solvents and did not melt at 290° C. The chlorides that dissolved in alcohol were fractionally crystallised from chloroform, and yielded two *chloromercuri*-o-*cresols.* The less soluble in chloroform was crystallised repeatedly from benzene, a mixture of benzene and ethyl acetate and finally from alcohol, and then melted constantly at 210° C. (decomp.). (Found—Hg, 57·8; C_7H_7OClHg requires : Hg, 58·4 per cent.) It dissolved readily in acetone or ethyl acetate, fairly readily in hot alcohol,

but sparingly in chloroform, benzene, or light petroleum; 100 g. of water dissolved 0·014 g. at 25° C. The *chloromercuri*-o-*cresol* that was more readily soluble in chloroform separated from this solvent in elongated plates and from aqueous alcohol in long slender needles, which decomposed to a red liquid at 183° C. (Found—Hg, 58·8; C_7H_7OClHg requires : Hg, 58·4 per cent.) One hundred grams of water dissolved 0·019 g. at 25° C.

Mercuration of Aniline and *p*-Toluidine

p-*Acetoxymercuriacetanilide* was prepared by the mercuration of aniline and subsequent acetylation of the less soluble portion of the product as described by Dimroth.[12] It melted at 224–225° C., whereas Dimroth gives the m.p. as 220–221° C.; 100 g. of water dissolve 0·142 g. at 25° C. On prolonged boiling with water, acetic acid is split off and a fairly readily soluble base formed, which separates as a somewhat oily mass on concentration and cooling; it is readily reconverted to the original substance on treatment with acetic acid.

o-*Acetoxymercuriacetanilide* was prepared by mercuration of acetanilide in boiling aqueous solution as described by Pesci[29] who, however, isolated only *p*-acetoxymercuriacetanilide (Dimroth[12]). The crude *p*-acetoxymercuriacetanilide that separated on cooling was removed, the filtrate shaken with ether to remove unchanged acetanilide, evaporated to dryness, and the residue crystallised from ethyl acetate. o-*Acetoxymercuriacetanilide* melts at 163° C.; Dimroth,[12] who obtained it by acetylation of *o*-acetoxymercurianiline, gives the m.p. as 156–158° C. (Found—Hg, 51·2%; $C_{10}H_{11}O_3NHg$ requires : Hg, 50·9 per cent.) It is fairly readily soluble in water, but on prolonged boiling behaves similarly to the *para* isomeride, yielding a base that is readily reconverted into the acetoxy compound on treatment with acetic acid.

2-*Acetoxymercuriacet*-p-*toluidide*, prepared as described by Vecchiotti[35], melted at 179° C. (corr.); Vecchiotti gives m.p. 175° C. It was recovered unaltered after boiling for five hours with water.

The method described by White[37] has been used to determine mercury in the organo-mercury compounds. It gives excellent results with all of the mono-mercurated compounds, but is much less reliable with the dimercurated compounds which have been examined, principally owing to the difficulty of decomposing these completely with the mixture of permanganate and sulphuric acid recommended.

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